

in the art, amplification and/or quantification need not necessarily occur to do genotyping. In these embodiments, the assay generally relies on the use of two (or more, in the cases of three base alleles or four base alleles) label probes, each of which has ETMs with different redox potentials (E_0) that can be distinguished in the assay. In this way homogeneous and heterogeneous alleles can be distinguished (the former being either all first label or all second label, and the latter showing two peaks at each label potential).

[0277] Thus the present invention provides “assay complexes” (referred to herein and in the cited patents as “hybridization complexes” when the targets are nucleic acids) that are formed as “sandwich assay complexes”, as depicted in the Figures of many of the cited patents. See for example FIG. 2B of U.S. Pat. No. 7,935,481, incorporated by reference (as are all of the Figures, accompanying legends and the associated specification descriptions). That is, only in the presence of the target analyte will the ETM(s) be present at the surface of the detection electrode, thus giving rise to a signal. Suitable ETMs are outlined in the cited cases (particularly useful in some embodiments are metallocenes, with ferrocene and ferrocene derivatives as defined in the incorporated patents), and all discussions relating to ETMs are specifically incorporated independently and optionally by reference.

[0278] The detection electrodes comprise capture binding ligands, preferably covalently attached. By “binding ligand” or “binding species” herein is meant a compound that is used to probe for the presence of the target analyte, which will bind to the target analyte. In general, for most of the embodiments described herein, there are at least two binding ligands used per target analyte molecule; a “capture” or “anchor” binding ligand (also referred to herein as a “capture probe”, particularly in reference to a nucleic acid binding ligand) that is attached to the detection electrode as described herein, and a soluble binding ligand (frequently referred to herein as a “signaling probe” or a “label probe” when the target is nucleic acid), that binds independently to the target analyte, and either directly or indirectly comprises at least one ETM.

[0279] Generally, the capture binding ligand allows the attachment of a target analyte to the detection electrode, for the purposes of detection. As is outlined in the cited patent list, attachment of the target analyte to the capture binding ligand may be direct (i.e. the target analyte binds to the capture binding ligand) or indirect (one or more capture extender ligands may be used).

[0280] In a preferred embodiment, the binding is specific, and the binding ligand is part of a binding pair. By “specifically bind” herein is meant that the ligand binds the analyte, with specificity sufficient to differentiate between the analyte and other components or contaminants of the test sample. However, as will be appreciated by those in the art, it will be possible to detect analytes using binding that is not highly specific; for example, the systems may use different binding ligands, for example an array of different ligands, and detection of any particular analyte is via its “signature” of binding to a panel of binding ligands, similar to the manner in which “electronic noses” work. The binding should be sufficient to allow the analyte to remain bound under the conditions of the assay, including wash steps to remove non-specific binding. In some embodiments, for example in the detection of certain biomolecules, the binding constants of the analyte to the binding ligand will be at least about 10^{-4} to 10^{-6} M^{-1} , with at least about 10^{-5} to 10^{-9} M^{-1} being preferred and at least about 10^{-7} to 10^{-9} M^{-1} being particularly preferred.

[0281] As will be appreciated by those in the art, the composition of the binding ligand will depend on the composition of the target analyte. Binding ligands to a wide variety of analytes are known or can be readily found using known techniques. For example, when the analyte is a single-stranded nucleic acid, the binding ligand is generally a substantially complementary nucleic acid. Alternatively, as is generally described in U.S. Pat. Nos. 5,270,163, 5,475,096, 5,567,588, 5,595,877, 5,637,459, 5,683,867, 5,705,337, and related patents, hereby incorporated by reference, nucleic acid “aptamers” can be developed for binding to virtually any target analyte. Similarly the analyte may be a nucleic acid binding protein and the capture binding ligand is either a single-stranded or double-stranded nucleic acid; alternatively, the binding ligand may be a nucleic acid binding protein when the analyte is a single or double-stranded nucleic acid. When the analyte is a protein, the binding ligands include proteins (particularly including antibodies or fragments thereof (FABs, etc.)), small molecules, or aptamers, described above. Preferred binding ligand proteins include peptides. For example, when the analyte is an enzyme, suitable binding ligands include substrates, inhibitors, and other proteins that bind the enzyme, i.e. components of a multi-enzyme (or protein) complex. As will be appreciated by those in the art, any two molecules that will associate, preferably specifically, may be used, either as the analyte or the binding ligand. Suitable analyte/binding ligand pairs include, but are not limited to, antibodies/antigens, receptors/ligand, proteins/nucleic acids; nucleic acids/nucleic acids, enzymes/substrates and/or inhibitors, carbohydrates (including glycoproteins and glycolipids)/lectins, carbohydrates and other binding partners, proteins/proteins; and protein/small molecules. These may be wild-type or derivative sequences.

[0282] In this embodiment, when the binding ligand is a nucleic acid, preferred compositions and techniques are outlined in U.S. Pat. Nos. 5,591,578; 5,824,473; 5,705,348; 5,780,234 and 5,770,369; U.S. Ser. Nos. 08/873,598 08/911,589; PCT Pub. Nos. WO 98/20162; WO 98/12430; WO 98/57158; WO 00/16089; WO 99/57317; WO 99/67425; WO 00/24941; WO 00/38836; WO 99/37819; and WO 99/57319; PCT Application Nos. PCT/US00/10903 and PCT/US00/20476; and related materials, all of which are expressly incorporated by reference in their entirety.

[0283] The method of attachment of the capture binding ligands to the attachment linker (either an insulator or conductive oligomer) will generally be done as is known in the art, and will depend on both the composition of the attachment linker and the capture binding ligand. In general, the capture binding ligands are attached to the attachment linker through the use of functional groups on each that can then be used for attachment. Preferred functional groups for attachment are amino groups, carboxy groups, oxo groups and thiol groups. These functional groups can then be attached, either directly or indirectly through the use of a linker as described in the list above.

[0284] In this way, capture binding ligands comprising proteins, lectins, nucleic acids, small organic molecules, carbohydrates, etc. can be added.

[0285] A preferred embodiment utilizes proteinaceous capture binding ligands. As is known in the art, any number of techniques may be used to attach a proteinaceous capture binding ligand to an attachment linker. A wide variety of techniques are known to add moieties to proteins.